(*Dlx5/6*). *Dlx5/6* induce expression of *Hand2*, a basic helix-loop-helix transcription factor. This pathway places *Hand2* at the center of a complex signaling cascade, but little is known of its function in mammalian craniofacial development since *Hand2*$^{-/-}$ embryos die around embryonic day (E) 10.5 from vascular failure. To bypass these defects, we created a conditional deletion of *Hand2* using a traditional Cre-*lox*P system. Using the *Wnt1-Cre* mouse line, we delete *Hand2* within all migrating NCCs. Mutant mice exhibit severe craniofacial defects including mandibular hypoplasia, a single incisor, aglossia, and loss of tympanic rings and Meckel's cartilage. These changes are preceded by aberrant maintenance of *Dlx5/6* expression in the distal mandibular arch and subsequent upregulation of *Runx2* expression. In vitro studies show that Hand2 is able to repress the Dlx5/6 enhancer, I56i. This suggests that Hand2 functions by repressing *Dlx5/6* expression within the distal midline. In its absence, *Dlx5/6* expression is maintained and results in expression of *Runx2* followed by the repatterning of distal tongue mesenchyme to bone.

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**Program/Abstract # 91**
**Gain-of-function in Ras signaling perturbs dental development in mouse and human**
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Ras/MAPK signaling is critical in animal development, and RTK signaling, which activates Ras signaling, is known to play an important role in tooth development. Our previous work has shown that increasing Ras/MAPK signaling by inactivating Sprouty genes adversely affects tooth morphogenesis. Here, we directly examined the effects of activating Ras/MAPK signaling in both humans and mice. Costello Syndrome (CS) is caused by a heterozygous de novo germline mutation in HRAS that results in a constitutively active Ras protein. We examined a cohort of CS patients and identified a number of craniofacial and dental anomalies. We found that a large majority of patients presented with pronounced enamel hypoplasia. Microcomputed tomography of exfoliated primary teeth from CS patients showed a significant decrease in enamel thickness compared to controls. We next examined the CS mouse model and found that the mice also had an enamel defect. Further inspection revealed disorganization of the ameloblasts in the mouse incisor. We are currently studying cell proliferation and polarity of the amelobasts in the mutant mouse incisors. In addition, we are using an ameloblast-like cell line to determine the effects of increased Ras/MAPK signaling on the behavior of the cells.

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**Program/Abstract # 92**
**The control of inner ear morphogenesis by Sprouty and Tbx1 genes in mouse models of 22q11.2 deletion syndrome**
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DiGeorge/Velo-caldio-facial/22q11 deletion syndrome (22q11DS) is one of the most common microdeletion disorders, characterized by severe malformations of many organ systems. Most of the patients have some form of hearing loss and about 10% have defects in the structure of their inner ears. However, while for some people the condition is severe, for others it is not, and it is not yet known what causes the differences between individuals. Haploinsufficiency for the *TBX1* gene has been linked to 22q11DS defects, including inner ear defects. We have recently identified another group of genes, the Sprouty (*Spry*) genes, that regulate inner ear development. Mice with loss of function mutations in the *Spry1* and *Spry2* genes have abnormally shaped cochleas and semi-circular canals. To determine whether these genes interact with *Tbx1* during ear development, we generated *Spry1-/-;Spry2-/-;Tbx1* embryos and examined their inner ear structures by paint-fill metod. *Spry1-/-;Spry2-/-;Tbx1* embryos have a more severe phenotype than *Spry1-/-;Spry2-/-* or *Tbx1* mutants. We found that the several signaling pathways implicated in inner ear development are deregulated in the *Spry1-/-;Spry2-/-* otic vesicle and that these effects are further enhanced by *Tbx1* haploinsufficiency. These data suggest that Sprouty genes have the potential to modify inner ear development in patients with 22q11DS.

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**Program/Abstract # 93**
**The Role of FGF Gradients in the Regulation of Early Limb Growth**
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While molecular biology can identify the molecular components regulating tissue growth, by itself it can not explain how embryos determine their shape and size. FGFs, which control cell proliferation, differentiation, migration and survival, are key molecules in embryonic morphogenesis. In this paper, we use a reaction-diffusion model for morphogen diffusion and a Glazier-Graner-Hogeweg multi-cell model to simulate numerically the role of FGF4 and FGF8 in regulating the early growth of the vertebrate limb. FGF diffusion, decay and secretion, and cell growth in response to FGF concentrations, determine the shape and size of limbs, and hence more generally, of tissues and organs during embryonic development. Physiologically reasonable values for FGF secretion, diffusion and decay grow a simulated limb with correct shape, size and antero-posterior asymmetry. We show that the limb mainly expands by growth of the distal domain which has high FGF concentrations and that the distalized expansion locks the region of high FGF concentration into the distal tip. We conclude that the interaction between growth and FGF gradients dominates regulation of the proximo-distal and antero-posterior outgrowth of the limb and the FGF distribution.

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**Program/Abstract # 94**
**The Limb Mesenchyme Recruitment Model for Patterning the Vertebrate Limb**
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